REMARKS

Claims 1-50 were pending in the application. Claims 20-50 have been canceled without prejudice, in view of the restriction requirement and Applicants' election of Group I, claims 1-19.

The specification has been amended to correct a typographical error and a factual error. First, the specification has been amended at page 30, line 18, to change the misspelled name "Zhoa" to "Zhao." Second, Applicants have deleted a factual misstatement in the specification regarding Mathur *et al.*, "Journal of Macromolecular Science-Reviews In Macromolecular Chemistry and Physics," C36:405-430, 1996 ("the Mathur reference," copy enclosed). Applicants originally submitted the Mathur reference in an Information Disclosure Statement mailed on July 31, 2000. The Examiner enclosed a copy of the initialed Form PTO-1449 with the Office Action mailed January 12, 2001 (Paper No. 11).

Applicants have made two deletions with respect to the Mathur reference. First, Applicants have deleted the citation to the Mathur reference from the parenthetical after the following sentence:

> Michael-type addition to conjugated unsaturated groups can take place in good to quantitative yields at room or body temperature and in mild conditions with a wide variety of nucleophiles.

Page 30, lines 13-15. Second, Applicants have amended the sentence at page 30, lines 18-20, as follows:

> Conjugated unsaturated groups, such as vinyl sulfones (Pathak, supra) ~~or acrylamides (Mathur, supra)~~, have been used to link PEG or polysaccharides to proteins through Michael-type reactions with amino- or mercapto-groups.

The reason for these changes is that the Mathur reference does not disclose using acrylamides to link polyethylene glycol or polysaccharides to proteins through Michael-type reactions with amino or mercapto groups. Rather, the Mathur reference discloses

polymerizing a precursor component containing an acrylamide in the presence or absence of another precursor component using redox polymerization, radiation-induced polymerization (*e.g.*, γ-ray-induced polymerization), or free-radical crosslinking. The Mathur reference also discloses polymerizing precursors containing acrylic acids and/or precursors containing methacrylic acids using photoinduced polymerization, redox polymerization, radiation-induced polymerization, or free-radical crosslinking. All of the polymerization reactions disclosed in the Mathur reference are initiated by the formation of free radicals. The free radicals propagate through the carbon double bonds of the precursor components resulting in a chain reaction of precursor components and the formation of a polymerized gel (see, for example, page 408, lines 2-6 and 22-24). The Mathur reference does <u>not</u> disclose, teach, or suggest nucleophilic addition reactions between a nucleophile and a conjugated unsaturated group.

No new matter has been added by these amendments. Furthermore, Applicants have enclosed another copy of the Mathur reference for the Examiner's review in light of these amendments, and specifically solicit any questions or comments the Examiner may have regarding the Mathur reference.

Rejection of Claims 1-19 under 35 U.S.C. § 112, ¶ 1

Claims 1-19 stand rejected as not enabled by the specification. In particular, the Office Action asserts that the specification "does not reasonably provide enablement for the generic class of methods of combining two or more 'precursors' by nucleophilic addition." Office Action at 4. The Office Action further asserts that the present specification "offers only relatively narrow guidelines in making and using only one class of nucleophile, those used to construct PEG derivatives used as hydrogels." *Id.* In support of this rejection, the Office Action states that the art (Hern *et al.*, J. Biomed. Mater. Res. 39:266-76, 1998) "teaches that PEG has several properties which are

essential for hydrogels. The compounds must not stick to cells, must be biodegradable, and must be able to be synthesized *in vivo*." *Id.* The Office Action concludes that:

> Lacking any further guidance from the specification the skilled artisan must perform the undue experimentation of developing synthesis protocols, testing cell adhesion, biodegradability etc. of the resulting polymers. Since the claims are not limited to uses of any kind, the skilled artisan must also uncover a reasonable use for these compounds.

Id. Applicants respectfully traverse this rejection.

Applicants respectfully assert that claims 1-19 are fully enabled by the specification. As an initial matter, Applicant note that the pending claims are directed to "polymerization which occurs through self selective reaction between a strong nucleophile and a conjugated unsaturated bond or a conjugated unsaturated group . . . ." The specification explains that "self selective" means that:

> a first precursor component of the reaction reacts much faster with a second precursor component of the reaction than with other compounds present in the mixture at the site of the reaction, and the second precursor component reacts much faster with the first precursor component than with other compounds present in the mixture at the site of the reaction. The mixture may contain other biological materials, for example, drugs, peptides, proteins, DNA, cells, cell aggregates, and tissues.

Page 8, lines 17-23. While thiols are used as the nucleophilic groups in a preferred embodiment of the claimed method, the specification also explains that amine nucleophilic groups may be used. *See* page 7, lines 20-23. Applicants further teach that:

> When the highest degree of self selectivity is desired in the methods of the invention, a thiol is the nucleophile of choice. When the highest level of selectivity is not required in the methods of the invention, an amine may be used as the strong nucleophile. Conditions utilized to complete the self selective reaction of the present invention can be altered to increase the degree of self selectivity, as provided herein. For example, if an amine is used as the strong nucleophile in the formation of a biomaterial by

> selection of an amine with a low pK, and the final precursor solution to be polymerized is formulated such that the pH is near the pK, the reaction of the unsaturation with the provided amine is favored and thus self selectivity is achieved.

Page 9, lines 1-9.

Methods of making nucleophilic precursors for use in the method of the invention are exhaustively described in the specification. As explained in the specification:

> the symbol **P** is employed to indicate the part of a molecule that lies between two reactive sites (telechelic sense) or is grafted with reactive sites (grafted sense). With telechelic polymers, **P** will lie between two strong nucleophiles, such as two thiols, or between two conjugated unsaturations (e.g., in the case of a PEG diacrylate or a PEG dithiol, **P** is a PEG chain). In the case of a PEG triquinone or trithiol, **P** is a three-armed, branched PEG. In the case of a block copolymeric acrylate-(lactic acid oligomer)-PEG-(lactic acid oligomer)-acrylate or quinone-(lactic acid oligomer)-PEG-(lactic acid oligomer)-quinone, **P** is the (lactic acid oligomer)-PEG-(lactic acid oligomer) block copolymer. In the case of a graft copolymer (e.g., polylysine-graft-(PEG acrylate) or polylysine-graft-(PEG quinone) or polylysine-graft-(PEG thiol)), in which the geometry of the polymer is as a bottle-brush with the tips of the bristles containing either the conjugated unsaturations or the strong nucleophile, **P** is polylysine-graft-(PEG). **P** can also present the reactive groups in the side chains: every polymer bearing alcohols or amines in the side chains is easily acrylated, for example, in order to present multiple conjugated unsaturated groups for the conjugate addition reaction. Polymers containing carboxylic acids can be derivatized to expose, for example, quinines groups. **P** need not be polymeric in the usual sense of the word.

Page 28, line 24 – page 29, line 15.

Thus, for example, the preparation of protein nucleophiles is described at page 42, line 14 – page 43, line 2:

> **P** may be a protein. The protein can be a naturally occurring or recombinant protein. In general terms, the recombinant proteins are any length amino acid material generated through recombinant DNA technology. Examples of components these can have include peptide sequences which encode degradation sites for proteases,

peptide sequences for other biological signals and non biointeractive sequences.

> Any naturally occurring protein can also be **P**. More specifically, a naturally occurring protein is composed of several **P**s which are separated by nucleophiles. For example, serum albumin, a 584 amino acid protein, contains 5.7 % cysteine, 9.9 % lysine and 3.1 % tyrosine. The amino acid sequences which occur between, for example, cysteine, tyrosine and lysine make up distinct **P**s. While albumin in its natural state may be less than useful for the purposes of cross-linking by conjugate addition reactions between conjugated unsaturations and thiols on the protein, albumin can be readily processed by reduction so as to form a poly(amino acid) with some or all of its cysteine residues free or it can be chemically derivatized to introduce multiple thiol groups.

Similarly, the preparation of peptide and polypeptide precursors is further detailed at page 29, lines 16-25, where it is stated that:

> In the case of a peptide, for example, YCXXXXXXCY (SEQ ID NO: 1) or CXXXXXXC (SEQ ID NO: 2), where C is the amino acid cysteine and X and Y are other amino acids, such that XXXXXX (SEQ ID NO: 3) could be a sequence that functions as a substrate for a protease such as collagenase, **P** is XXXXXX. The length of XXXXXX or the number of X (e.g., Xn) can be any length or number (n=0).

The specification further explains that:

> In some instances, **P** may be a peptide or a polypeptide, where the nucleophile is the amino acid cysteine, resulting in peptides of structures similar to H_2N-CXXXXXCXXXXXC-COOH (SEQ ID NO: 4) or H_2N-CXXXXXC-COOH (SEQ ID NO: 5), where C is the one-letter representation of cysteine, and X represents any amino acid except cysteine, in one embodiment, or Acetyl-NH-YXXXXXYXXXXXY-COOH (SEQ ID NO: 6) where Y is the one-letter representation of tyrosine, and X represents any amino acid except cysteine or tyrosine, in another embodiment. The length of XXXXX (SEQ ID NO: 7) or the number of X (e.g., Xn) can be any length or number (n=0).It is particularly useful when the sequences in the domains shown as XXXXX above are substrates for enzymes that are involved in cell migration (e.g., as substrates for enzymes such as collagenase, plasmin or elastase), although the domains need not be limited to these. One such particularly useful sequence, as a substrate for the enzyme plasmin,

> is described in the examples. A variety of such peptides may be learned from a study of the literature of these enzymes.

Page 43, lines 4-18. Peptides suitable for use with the claimed invention are further described in Tables 1, 2, and 3. These teachings are further buttressed by Examples 2 and 8, which describe the use of a peptide (SEQ ID NO 58) linked nucleophile to form a gel by conjugate addition (Example 2) and the formation of a pH sensitive gel using a peptide (SEQ ID NO 67) in a conjugate addition reaction (Example 8).

The specification is replete with other examples of nucleophiles that may be used in the claimed method. *See also* Example 15 (describing preparation of materials that are responsive to environmental conditions).

Similarly, the specification describes a plethora of conjugated unsaturated structures for use in the claimed method. For example, in the section entitled "Conjugated unsaturated structures," page 31, line 10 – page 38, line 20, twenty exemplary structures are described for use with the claimed method. In addition, the specification teaches that:

> Reactive double bonds can be conjugated to one or more carbonyl groups in a linear ketone, ester or amide structure (**1**, **2**) or to two in a ring system, as in a maleic or paraquinoid derivative (**3**, **4**, **5**, **6**, **7**, **8**, **9**, **10**). In the latter case the ring can be fused to give a naphthoquinone (**6**, **7**, **10**) or a 4,7-benzimidazoledione (**8**) (Pathak, supra) and the carbonyl groups can be converted to an oxime (**9**, **10**). The double bond can be conjugated to a heteroatom-heteroatom double bond, such as a sulfone (**11**), a sulfoxide (**12**), a sulfonate or a sulfonamide (**13**), a phosphonate or phosphonamide (**14**). Finally, the double bond can be conjugated to an electron-poor aromatic system, such as a 4-vinylpirydinium ion (**15**). Triple bonds can be used in conjugation with carbonyl or heteroatom-based multiple bonds (**16**, **17**, **18**, **19**, **20**).

Page 31, line 17 – page 32, line 1.

The specification also provides examples of several precursor components, in addition to polyethylene glycol polymers, that may be used in the claimed method:

P can be synthetic hydrophilic polymers, synthetic hydrophobic polymeric liquids, synthetic hydrophobic polymers that are soluble in solvents of acceptable toxicity or biological influence for the envisioned application, biosynthetic proteins or peptides, naturally occurring proteins or processed naturally occurring proteins, or polysaccharides.

* * *

[T]he synthetic polymer **P** can be poly(ethylene glycol), poly(ethylene oxide), poly(vinyl alcohol), poly(ethylene-co-vinyl alcohol), poly(acrylic acid), poly(ethylene-co-acrylic acid), poly(ethyloxazoline), poly(vinyl pyrrolidone), poly(ethylene-co-vinyl pyrrolidone), poly(maleic acid), poly(ethylene-co-maleic acid), poly(acrylamide), or poly(ethylene oxide)-co-poly(propylene oxide) block copolymers This is not an exhaustive list as other hydrophilic polymers could also be used.

P can also be copolymers, block copolymers, graft copolymers, or random copolymers. Blocks, which are polymerized on the ends of the hydrophilic polymers, can be composed of, for example, lactic acid, glycolic acid, epsilon-caprolactone, lactic-co-glycolic acid oligomers, trimethylene carbonate, anhydrides, and amino acids, for example, to confer degradability by hydrolytic or enzymatic means. This list is not exhaustive; other oligomers may also be used for block copolymers.

Random copolymers can be based on vinyl alcohol, such as poly(N-vinylpyrrolidone-co-vinyl alcohol) or poly(ethylene-co-vinyl alcohol), with different compositions, can be derivatized with conjugated unsaturated groups, such as acrylates, benzoquinones, naphthoquinones and others. The vinyl alcohol copolymers can be functionalized with $(CH_2)_n$ COOH groups by reaction with ω-bromo-carboxylic acids. The resulting polymers or acrylic or methacrylic acid copolymers can be used for the attachment of quinone groups.

Page 41, lines 9-13; page 41, line 15 – page 42, line 8. Accordingly, Applicants respectfully submit that the current specification provides exhaustive guidance concerning the precursors which may be used for the self selective reaction of the claimed invention.

As concerns the Hern reference, Applicants first note that the reference is concerned only with the formation of hydrogels through photopolymerization, *i.e.*, free radical initiated polymerization. As such, Hern is reporting the properties of the hydrogels prepared by photopolymerization. In particular, Hern *et al.* state, at page 266, that:

> Polyethylene glycol (PEG) has been employed as a biomaterial because of its remarkable nonadhesivity towards proteins and, hence, toward cells.
>
> * * *
>
> PEG diacrylate hydrogels adhere to tissue surfaces upon which they are polymerized, presumably by flow of precursor into tissue texture or by diffusion of precursor into the extracellular matrix to form, after polymerization, either mechanical interdigitation or an interpenetrating polymer network, respectively. The polymerized hydrogels are relatively nonadhesive to cells at the free surface, however, presumably due to poor protein adsorption to the hydrophilic, nonionic material surface.

Applicants respectfully submit that the Hern reference does not support the proposition in the Office Action that "PEG has several properties which are <u>essential</u> for hydrogels." Office Action at 4 (emphasis added). If the Examiner believes that some other passage from the Hern reference supports this proposition, Applicants respectfully request that the Examiner provide Applicants with that specific text. Rather, Applicants submit that the Hern reference merely acknowledges that adhesive peptides may be incorporated into non-adhesive PEG hydrogels. This is but one aspect of the present invention. As explained in Applicants' specification:

> One strong benefit of the use of the addition reactions described herein is that other bioactive biofunctional groups can be incorporated into the biomaterial, for example, to provide sites for binding of adhesion-promoting receptors on the cell surface or sites for growth factor binding.

Page 51, lines 20-24. Applicants further describe "a variety of adhesion-promoting peptides" which may be used in accordance with the method of the invention. *See* Table 4 and SEQ ID NOS: 39-49.

Finally, the Office Action asserts that because "the claims are not limited to uses of any kind, the skilled artisan must also experiment to uncover a reasonable use for these compounds." Office Action at 4. First, to the extent the Office Action seeks utility for the biomaterial made according to the methods of claims 1-19, Applicants refer the Examiner to the section of the specification entitled, "Biomedical Applications for Hydrogels," page 24, line 1 – page 28, line 22, and respectfully submit that this section provides a sufficient number of uses for the biomaterial made by the claimed method. Second, Applicants respectfully submit that the claims are "not limited to uses of any kind" because they are "method of making" claims, not "method of use" claims.

For all the foregoing reasons, Applicants respectfully submit that the rejection of claims 1-19 under 35 U.S.C. § 112, ¶1 should be withdrawn. Far from performing undue experimentation, the skilled artisan need only read Applicants' specification to determine appropriate synthetic protocols, cell adhesion, and biodegradability of the resulting biomaterials. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

Rejection of Claims 1-19 under 35 U.S.C. § 103(a)

Claims 1-19 stand rejected under 35 U.S.C. § 103(a) as obvious in view of the Hern reference. In particular, the Office Action asserts that:

> Hern teaches the two precursors for PEG, including a nucleophile. See Hern p. 266. Hern also teaches the claimed strong nucleophiles comprising thiol groups, self selective reactions, acrylate unsaturated groups, three functionalities, an adhesion site,

> synthesis within cells or tissues. See, for example, Hern, p. 267, Fig. 1; p. 268, col. 2; "acrylation" p. 269, col. 2.
>
> The claims differ from Hern in the recitation "wherein the functionality of each component is at least two." However, it would have been *prima facie* obvious for one of ordinary skill in the art at the time the invention was made to use monomers having two functionalities for the reason, explicitly set forth in Hern, of promoting polymerization.

Office Action at 5. Applicants respectfully traverse this rejection.

As discussed above, Applicants claimed invention is directed to a method of making a biomaterial by polymerization through a self selective reaction. In contrast, Hern teaches the use of photopolymerization using cross-linking chemistries that are not self selective, *i.e.* linking a peptide via a primary amine to a polymer via an amine reactive N-hydroxysuccinimidyl ester. In addition, contrary to the statement in the Office Action, Hern does not teach, suggest, or disclose the concept of strong nucleophiles – let alone the use of strong nucleophiles in a self selective reaction. This is primarily because the coupling reaction in Hern is a condensation reaction, *not* an addition reaction. Thus, there is no mention of a "thiol" or any thiolated material in Hern. Accordingly, for all the foregoing reasons, Applicants respectfully request that the rejection of claims 1-19 as obvious over Hern be withdrawn.

CONCLUSION

Applicants submit the pending claims are in condition for allowance and respectfully request prompt and favorable action. If an interview with Applicants' attorney would expedite prosecution, the Examiner is invited to call the undersigned at 617-428-0200.

Enclosed is a petition to extend the period for replying for three months, to and including July 12, 2001. If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: July 11, 2001

Timothy J. Douros
Reg. No. 41,716

Clark & Elbing LLP
176 Federal Street
Boston, MA 02110
Telephone: 617-428-0200
Facsimile: 617-428-7045

21559
PATENT TRADEMARK OFFICE

F:\50154\50154.002002 Reply to Exam. Action 1-12-01.wpd

Version with Markings to Show Changes Made

Michael-type addition to conjugated unsaturated groups can take place in good to quantitative yields at room or body temperature and in mild conditions with a wide variety of nucleophiles (Pathak, supra; ~~Mathur et al., Journal of Macromolecular Science-Reviews In Macromolecular Chemistry and Physics," C36:405-430,1996;~~ Moghaddam et al., Journal of Polymer Science: Part A: Polymer Chemistry 31:1589-1597, 1993; and Zhao~~Zhoa,~~ supra). Conjugated unsaturated groups, such as vinyl sulfones (Pathak, supra) ~~or acrylamides (Mathur, supra)~~, have been used to link PEG or polysaccharides to proteins through Michael-type reactions with amino- or mercapto-groups.